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The 22-kDa Antigen in Optic Nerve and Retinal Diseases

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Objective: Patients with unexplained visual loss were evaluated for the possibility of immunologic involvement. Antibody reactions were sought that might identify a common indication of retinal hypersensitivity.

Methods: The enzyme-linked immunosorbent assay (ELISA) and Western blot analysis were used to identify autoantibody reactions with retina and optic nerve components. Comparisons were made with the autoantibody reaction of normal subjects and patients with recognized forms of retinal decay: macular degeneration, retinitis pigmentosa, diabetic retinopathy, and paraneoplastic retinopathy.

Results: Eight patients, one man and seven women, were found to produce an autoantibody reaction with retina and optic nerve, including a novel 22-kDa neuronal antigen present within the retina and optic nerve. One of the eight had retinopathy associated with melanoma (MAR Syndrome). Seven of the eight patients had electroretinogram abnormalities, varying from mild to severe. Six displayed features of optic atrophy. One patient with progressive visual loss had visual function stabilized after immunosuppressive therapy.

Conclusions: In the eight cases described, unexplained visual loss was associated with autoantibody reactions with retina and optic nerve, including a common antibody reaction with a 22-kDa neuronal antigen found in the retina and optic nerve. All the patients had either an abnormal electroretinogram or optic atrophy. Six patients had both. The 22-kDa immunologic marker may not be directly involved in the patient's vision loss, but rather may be related to a nonspecific destruction of retina and optic nerve. However, the marker may be useful in identifying a specific subgroup of patients for further analysis.

Key Words: 22-kDa Antigen—Autoantibody—Non-cancer-associated retinopathy—Optic neuropathy—Retinopathy.

Indications of ocular hypersensitivity developing in patients with vision loss from unknown cause may suggest the possibility of autoimmune involvement. Several forms of visual loss implicate immunologic reactions, ranging from the more obvious forms of inflammatory

uveitis to the less evident paraneoplastic retinopathies (1-21). From an immunologic standpoint, the composition of the eye is exceedingly complex, consisting of a multitude of different proteins, carbohydrates, and lipids working in concert to transmit light-induced signals to the brain. If hypersensitivity plays a part in some forms of visual loss, there are many potential targets for autoantigenic involvement. Studies of the increasing numbers of reports of cases of visual loss attributed to allergic reactions have focused on a limited number of potential autoantigens, with interest centering on single retinal proteins. Subgrouping patients according to their collective immunologic reactions provides a format for organizing investigations and providing a framework for further inquiry. Applying this approach, we identified eight patients who had declining and unexplained loss of vision while producing autoantibodies reactive with a low-molecular-mass 22-kDa neuronal antigen present within the retina and optic nerve.

Although the patients described in this report did not have a common clinical entity, they all had retinal or optic nerve dysfunction, or both. We had looked at both normal subjects and those with various retinal and optic nerve diseases and had not found the 22-kDa antigen previously.

METHODS

This laboratory functions as a source of reference for physicians evaluating patients who are experiencing possible vision loss due to autoimmune reactions. Over the past 14 years, antibody reactions have been assayed in more than 2000 samples of sera of patients with a wide variety of recognized and mysterious forms of vision loss. Enzyme-linked immunosorbent assay (ELISA) and Western blot analyses are used to identify unusual antibody reactions with the antigens available in saline extracts of ocular tissues. Antibody reactions are compared with those of a control group consisting of sera from normal subjects and patients with recognized ocular abnormalities: retinitis pigmentosa, diabetic retinopathy, macular degeneration, and paraneoplastic retinopathies. The purpose is to identify distinct, single neuronal antigens involved in subgroups of patients. This approach enables the grouping of retinopathy patients according to their common immunologic reactions with single ocular proteins.

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Enzyme-Linked Immunosorbent Assay

This procedure is used to identify abnormal concentrations of antibody reactions with the solubilized antigens of bovine ocular tissues and has been detailed in our previous studies (2,19).

Western Blot Analyses

Applying the methods we have described before (10), extracts of ocular tissues are separated electrophoretically and transferred to nitrocellulose, strips of which are probed with the patient's antibodies. We routinely assay new samples of sera at a dilution of 1:200 and compare the outcome with sera from the reference control group. Sera showing antibody reactivity in the range of 23-kDa are subjected to further analysis using the recombinant 23-kDa cancer-associated retinopathy retinal antigen (CAR), recoverin (20), based on our knowledge that retinal proteins other than the 23-kDa CAR autoantigen also migrate to this same location in Western blot analyses of whole retina.

RESULTS

Eight patients, referred to us because of unexplained vision loss, had an autoantibody reaction to retina and optic nerve. These patients were further characterized by an unusual antibody reaction on Western blot analysis with a single 22-kDa neuronal antigen derived from the retina and optic nerve. The results of evaluation of their sera on the ELISA against standardized extracts of bovine retina and optic nerve are shown in Table 1.

In our laboratory, our studies are controlled by reference to normal human pooled serum, which reacts in the ELISA with titers approximating 1:100 on extracts of bovine retina and optic nerve (Table 1).

In addition, our studies are controlled by references made to patients with recognized systemic diseases such as diabetes, with and without laser therapy, macular degeneration, retinitis pigmentosa, multiple sclerosis, optic neuritis, and the 23-kDa CAR syndrome. Although pa-

tients from our comparison control group can be shown to produce varying autoantibody reactions with retina and optic nerve components, none was found to contain antibodies reactive with the 22-kDa neuronal antigen. A summary of the eight cases outlined here in detail is shown in Table 2.

Case 1

A 54-year-old woman reported central blurring of vision and occasional flickering lights in her left eye. Her initial visual acuity was 20/25 OD and 20/40 OS. Gradual visual loss continued to develop asymmetrically in both eyes over the next several years with marked reduction of electroretinogram (ERG), progressive optic atrophy, and vascular changes in her fundus.

Three years later, her serum was found to have an antibody reaction on ELISA of 1:400 against retina and 1:800 against optic nerve. In addition, on Western blot analysis, she had an antibody reaction to a unique 22-kDa neuronal antigen derived from the retina and optic nerve.

She had complete ophthalmologic, neurologic, and medical evaluations performed at several major universities throughout the United States, without having a clear cause defined for her progressive visual loss. No medical or neurologic abnormalities could be uncovered except to account for her progressive optic neuropathy and retinopathy.

Because of the antibody reaction against retina and optic nerve and the unique 22-kDa neuronal antigen, she underwent therapy with intermittent intravenous pulse steroids, and she continued to take oral steroids over several years. Finally, triple therapy was prescribed (cyclosporine, azathioprine, and prednisone; Table 3), which has been previously reported to help in serpiginous choroiditis (22). On a modified version of this regimen, her visual loss has stabilized over the past several years with visual acuity in the right eye of 20/70 and in the left eye of bare hand motion that is associated with a markedly reduced ERG in both eyes. In addition, she has moderate optic atrophy in the right eye with marked optic atrophy in the left eye. Her maculae show retinal pigment epithelial changes in the right eye with a macular hole in the left eye. In addition, she has sheathing of her vessels with bone spicule-like pigmentary deposits along blood vessels in her left eye with bone spiculating-type changes in the superior retina of both eyes. She has also had intermittent fine vitreous cells in both eyes over the years. A complete accounting of the numerous events in her ophthalmologic course is chronicled in Table 3.

Case 2

In 1981, a 65-year-old man described progressive visual loss of approximately 2 years' duration in both eyes. The patient was otherwise healthy with no known systemic disease. An ERG performed in 1987 had markedly reduced photopic and scotopic potentials. Visual evoked potentials were markedly reduced in amplitudes in both eyes. Dark-adaptation curves were mildly abnormally in both eyes. Medical history was significant only for a history of hypertension.

TABLE 1. ELISA: Evaluation of initial comparative antibody reactions on standardized extracts of bovine retina and optic nerve

	Retina	Optic nerve	
Case:			
1.	1:400	1:800	OA
2.	1:400	1:400	OA
3.	1:400	1:200	No OA
4.	1:800	1:200	OA
5.	1:400	1:200	No OA
6.	1:400	1:400	OA
7.	1:400	1:100	OA
8.	1:400	1:200	OA

Controlled normal pooled serum: retina <1:100; optic nerve <1:100

OA, optic atrophy; ELISA, enzyme-linked immunosorbent assay.

TABLE 2. 22 kd antigen

Case	Age of onset	Sex	Affected eye	Last recorded V.A.	Retina	Optic nerve	ERG	Systemic illness	Progressive visual loss
Case 1	Age 41	Female	OS > OD	20/70 OD H.M. OS	Vascular sheathing pigment along blood vessels	Moderate optic pallor OU	Moderate to severely abnormal OU	None	Yes: OS > OD Progressive 10 yrs. Stable with treatment 4 yrs
Case 2	Age 65	Male	Equal OU	20/200 OU	RPE atrophy, narrow blood vessels	Moderate optic pallor	Markedly abnormal OU	None	Probable slowly progressive
Case 3	Age 22	Female	OS > OD	20/40 OD C.F. OS	Massive geographic atrophy centrally with RPE hyperplasia OS > OD	Normal OU	Slightly reduced and delayed OU EOG abnormal OD, normal OS	None	22 years ago five years progressive visual loss OS; Mild loss OD 10 years
Case 4	Age 2	Female	OU	20/400 OU	Narrow blood vessels, bone spicules	Moderate optic pallor OU	Extinguished OU	"Viral Syndrome" with onset of visual loss	Stable
Case 5	Age 48	Female	OD	20/20 OD 20/15 OS	OD mild vessel attenuation, OS normal	Normal OU	OD extinguished Normal OS	None	2 years visual loss OD
Case 6	Age 43	Female	OD	C.F. 1 Foot OD 20/20 OS	Normal OU	Severe pallor OD Normal OS	Normal OU Slight reduction OD	None	Stable visual function OD 11 months
Case 7	Age 46	Female	OU	1/200 OU	Severe vessel attenuation OU	Moderate pallor OU	a wave slightly reduced, b wave absent OU	Metastatic cutaneous malignant melanoma	Progressive visual loss OD 16 months
Case 8	Age 49	Female	OU	20/200 OU	Slight vessel attenuation Focal yellow deposit in macula	Mild to moderate pallor OD Moderate pallor OS	a wave slightly reduced, b wave almost extinguished OU	None	Progressive over 6 years OU

V.A., visual acuity; ERG, electroretinogram; OS, left eye; OD, right eye; OU, both eyes; RPE, retinal pigment epithelium; H.M., hand motion; C.F., count fingers; EOG, electro-oculogram.

In 1991, visual acuity was 20/70 OD and 20/80 OS. Color vision was reduced in both eyes, and the patient was unable to identify any of the six Hardy-Rand-Rittler screening plates. Humphrey visual field testing (Humphrey, San Leandro, CA) showed scattered superior central scotomas in both eyes. Fundus examination revealed 2 to 3+ optic pallor of both optic nerves with narrowing

of the blood vessels and retinal pigment epithelial atrophy, but no bone spicules were seen. The patient's serum showed an antibody reaction on ELISA, 1:400 against retina and 1:400 against optic nerve (Table 1). Western blot analysis identified an antibody reaction with a unique 22-kDa neuronal antigen, present in the retina and optic nerve. Photopic and scotopic ERG at that time was

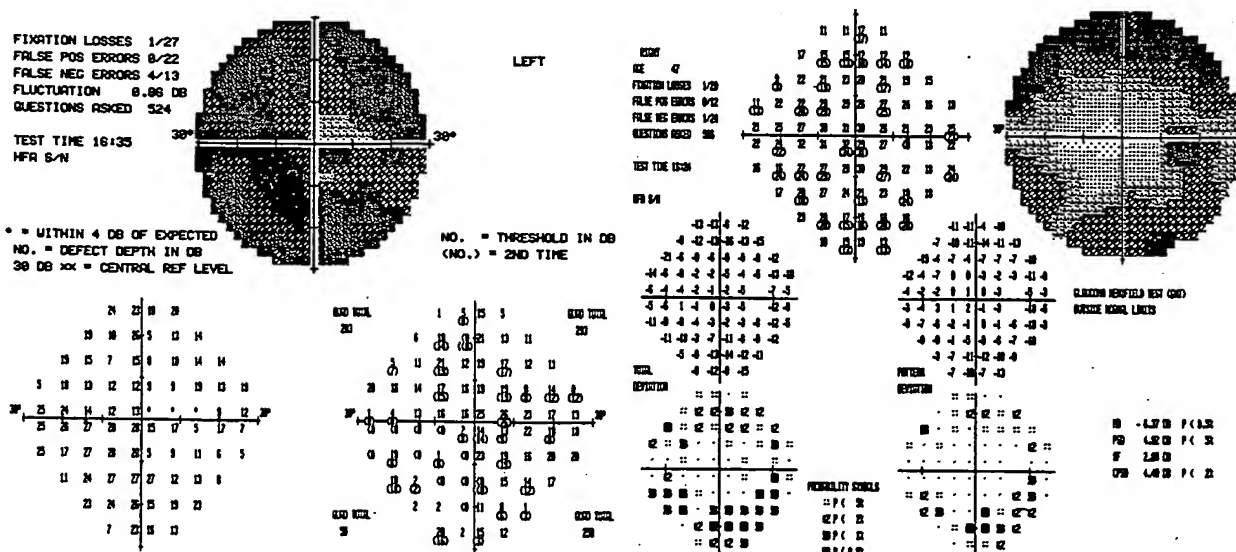


FIG. 1. Case 1: Humphrey (San Leandro, CA) visual field (June 4, 1987), right and left eyes. Note mean deviation of -6.37 dB OD. Size V test object used in the left eye.

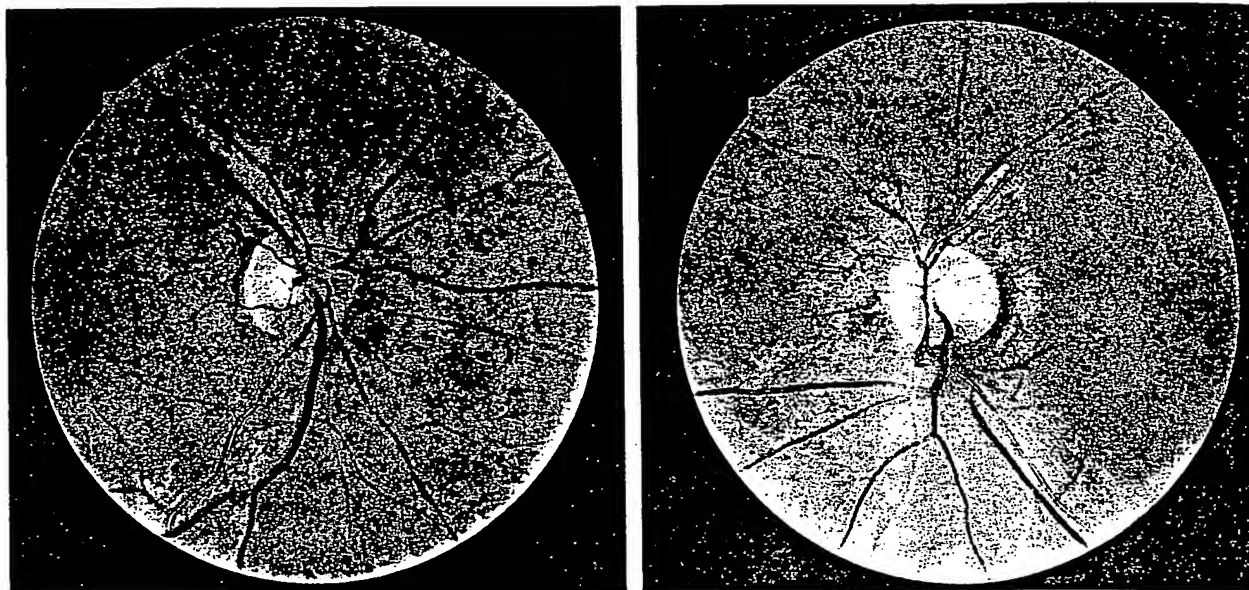


FIG. 2. Case 1: Right eye (on left) and left eye (on right). Note narrowed blood vessels and mild temporal pallor left eye > right eye.

markedly abnormal in both eyes, similar to the ERG of 1987. In 1995 at 79 years of age, the patient reported no real change in his vision, and he had enjoyed good health. However, his best corrected visual acuity had declined to 20/200 in each eye. The Humphrey field in the right eye showed further progression of the field loss with a larger central scotoma than in 1991. The left eye again showed a superior depression and greater central scotoma (Fig. 6). The Goldmann fields also suggested a central scotoma. Examination of the fundus in the right eye showed 2+ optic nerve pallor with choroidal atrophy around the macula. The vessels appeared slightly narrow. The left eye showed 2+ to 3+ optic nerve pallor with some choroidal atrophy around the macula (Fig. 7). His serum no longer contained antibodies reactive with the

22-kDa retinal antigen, and there was no longer a reaction on ELISA to retina or optic nerve. We have not seen the patient in the intervening 4 years. Because he was unaware of any change in his vision, we do not know when the progression began or when exactly his serum lost reactivity with the 22-kDa neuronal antigen.

Case 3

A 44-year-old woman described progressive visual loss. She had had poor vision since early adulthood. When pregnant with her only child at the age of 22, she had noted a relative loss of central vision in her right eye. A similar condition subsequently developed in her left eye. The visual loss progressed rapidly over 5 years to count fingers in the left eye. She was evaluated 15 years previously and was advised that she had "sclerosis." Over the past 10 years, the vision in her right eye had decreased. She had associated loss of night vision and, more recently, poor color vision. Her medical history was unremarkable. There was no family history of any hereditary disease. Furthermore, both parents and four of eight siblings were examined, and no evidence of disease was encountered.

Examination revealed visual acuity of 20/40 in the right eye and count fingers at 2 ft in the left eye. On the Ishihara color plates she identified only 4 of 15 plates in her right eye. Goldmann visual fields showed dense central scotomas in both eyes with peripheral scattered scotomas. No vitreous cells were seen. The fundus showed a massive area of geographic atrophy centrally with marked pigment disruption throughout the fundus, almost extending to the equator. The left eye also showed a massive area of geographic atrophy centrally with exposed choroidal vessels centrally and marked retinal pigment epithelial atrophy and hypertrophy peripherally (Fig. 8). The ERG was slightly reduced and delayed in both eyes. Electro-oculogram was abnormal in the right

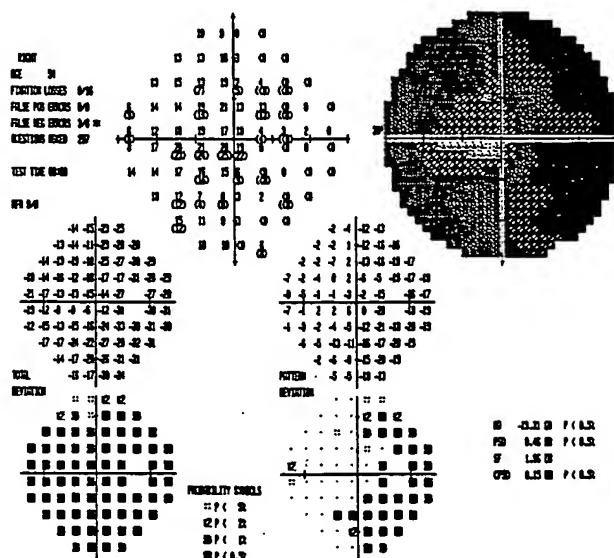


FIG. 3. Case 1: Humphrey (San Leandro, CA) visual field (April 5, 1994), right eye. Note mean deviation of -19.3 dB.

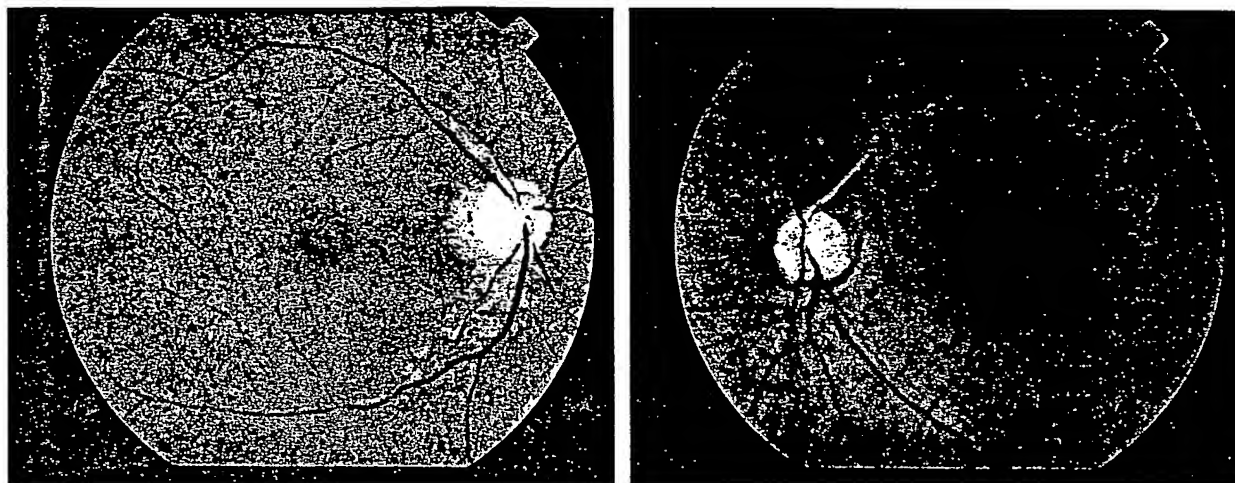


FIG. 4. Case 1: Fundus photograph (January 10, 1995), right eye (on left) and left eye (on right). Note pallor of both optic discs, left eye > right eye. Also note the narrowed blood vessels and sheathing, right superior temporal artery in the left eye not seen in Fig. 2.

eye and normal in the left eye. The dark-adaptation curve was elevated in the right eye and normal in the left.

The patient's serum showed an antibody reaction on ELISA, 1:400 against retina and 1:200 against optic nerve (Table 1). Western blot analysis identified an antibody reaction with a unique 22-kDa neuronal antigen, present in the retina and optic nerve.

Case 4

A young girl just short of her third birthday had been well except for a history of recurrent ear infections. Ten days before hospital admission, a fever developed with vomiting, cough, and rhinorrhea. Her family doctor noted a right ear and throat infection and prescribed amoxicillin. The vomiting subsided, and she seemed to recover. Her medical history was otherwise unremark-

able except for chicken pox 4 weeks earlier and a diphtheria-pertussis-tetanus immunization 12 days before admission. Three days before admission, the parents described her as "staring through objects." She would miss objects when reaching for them and appeared dazed. On admission, she was afebrile with normal findings in general and neurologic evaluations. Ophthalmologic examination found poor ability to fix and follow. Her optokinetic responses were poor. There was no evidence of vitritis. The optic nerves were normal, and macular edema was present. There was no evidence of scleritis. Oculomotor function was normal. Pupillary reflexes seemed sluggish to light. An ERG was almost flat (95–98% reduction to all stimuli). Therapy with intravenous steroids was prescribed, followed by an oral regimen, but there was little change in her visual function. A follow-up ERG 8 months later showed no change. Titers for herpes, histoplasmosis, toxoplasmosis, antinuclear antibody, and rheumatoid factor were negative. The subsequent course was wholly unremarkable.

Four years later, the patient's visual acuity was approximately 20/400 OU. The patient always tended to fixate eccentrically with her eyes in up-gaze. Pupillary reflexes were normal. She had slight exotropia, and slit lamp examination results were normal. Dilated retinal examination showed bilateral optic atrophy with mild retinal blood vessel attenuation and some bone spicule pigmentation throughout the peripheral retina in each eye (Fig. 9). There was chronic macular pigment stippling with overlying internal limiting membrane irregularities that appeared to be secondary to the original process. Initial acute neuroretinitis with a nonprogressive pseudo-retinitis pigmentosa, possibly related to varicella or another virus, was postulated. Five years after the initial illness, the patient's serum showed an antibody reaction on ELISA, 1:800 against retina and 1:200 against optic nerve (Table 1). Western blot analysis identified an antibody reaction with a unique 22-kDa neuronal antigen, present in the retina and optic nerve.

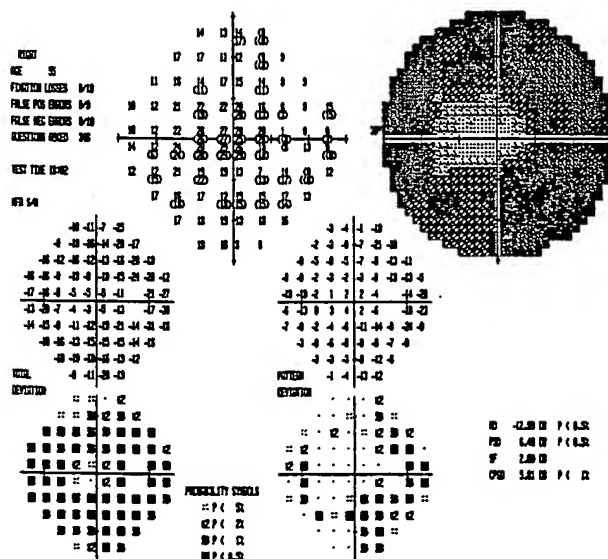


FIG. 5. Case 1: Humphrey (San Leandro, CA) visual field (September 7, 1995), right eye. Note mean deviation of -12.99 dB.

TABLE 3. Case 1 study summary

Date/institution	Best corrected VA	Visual field	Fundus examination	ERG	Laboratory values	22 KD and ELISA/ to bovine retina and ON	Treatment
3-24-84/ Cleveland, Ohio	OD 20/25 OS 20/40		Possible phlebitis of peripheral retinal vessels		MRI normal x2 VEP abnormal OS Collage vascular and neurologic evaluation negative		
5-1-85/Wilmer Institute	OD 20/25 OS 20/400				CSF TlgG, no oligoclonal bands or myelin basic protein. Collagen vascular. MRI, CT all neg. VEP abnormal OS		
7-2-85/Univ. of Pittsburgh	OD 20/25 OS 20/200		Narrowed vessels with mild sheathing OD; ON normal. OS ON pallor				
3-11-87/Bascom-Palmer Eye Institute	OD 20/40-2 OS 10/400		OD minimal ON pallor OS ON diffuse pallor	OD reduced B-wave OS marked reduction B and A wave		ELISA test: Retina 1/400 ON 1/800	After Elisa results, pred. 150 mg with taper over weeks
6-4-87/UC Davis	OD 20/40 OS 10/400	OD MD -6.37 dB OS size 5 diffuse loss (Fig. 1)	OD ON slight pallor OS ON moderate pallor Narrowed vessels and rare vitreous cells, OU (Fig. 2)	Marked reduction A&B wave much worse OS > OD	MRI, collagen vascular, rheumatologic and cancer evaluation all negative	Retina and optic nerve antibody titers normal, OU	Normal antibody titers following previous prednisone therapy
2-89/National Eye Institute	OD 20/80 OS CF		Narrowed blood vessels OS; ON pallor, OD				Chorioretinal biopsy recommended but declined
3-89/Bascom-Palmer Eye Institute	OD 20/80 OS LP			More reduction ERG OS > OD			
4-20-90/UC Davis	OD 20/60 OS HM	Worsening of HVF	OD-ON mild pallor OS-ON moderate pallor, sheathing of vessels superiorly, OU; RPE macular changes and fine vitreous cells OU	Progressive worsening of ERG OS > OD		Antibody titers ↑ to retina and optic nerve	
7-1-90 to 12-91/ UC Davis		VF stable with steroids		ERG stabilizes with pulse steroids	ANA positive 1/80 Serum complement slightly elevated	No retinal or ON antibodies following steroids	IVMP 1 gm, 3 days, prednisone 80 mg gradually tapered; intermittent IVMP
1-92 to 1-93/ UC Davis					Rheumatologic evaluation, Lyme titer negative.	Retinal and ON antibodies elevated before Triple Therapy	Triple Therapy: Cyclosporin, Imuran, & Pred. x 1 week (ref 22) stopped due to side effects
10-93 to 11-93/ UC Davis	OD 20/60 OS 20/400		Optic pallor OS > OD cellophane maculopathy narrowed blood vessels, bone spicules superonasally, 2+ vit. cells, OU	Marked vessel sheathing superiorly OS and nerve fiber layer hemorrhage off disk OS	MRI multiple times, tiny unidentified bright objects (UBOs) right frontal and right cerebral peduncle		
4-94 to 1-95/ UC Davis	OD 20/70 OS HM	5/94 worsening HVF MD OD -19.31 dB (Fig. 3) On 6/94 HVF MD OD improved to -15.3 dB w/ Rx	Similar eye findings, but increasing pallor. Note sheathing OS (Fig. 4)	Deterioration OD with no change OS			Give IVMP then modified Triple Therapy: Cyclosporin 100 mg/ qid; Imuran 100 mg, 75 mg. qod; Prednisone 20 mg (Pred stopped 6/94)
1-95 to 3-97 UC Davis	OD 20/70 OS HM	HVF stabilize with MD -12.99 dB 9/95 (Fig. 5)	Similar eye findings; new findings, macular hole, OS	ERG 3/18/97; stable compared with previous ERG		Serum free of antibodies to retina and ON	Current therapy 3/97 Cyclosporin 100 mg qd, Imuran 50 mg. alternating with 75 mg qd & Vit. E 1600 units q.d.

CF, count fingers; HM, hand motion; MD, mean deviation; IVMP, intravenous methylprednisolone; dB, decibels HVF units of light intensity measurement; HVF, Humphrey visual field; ON, optic nerve; Rx, treatment; MRI, magnetic resonance imaging.
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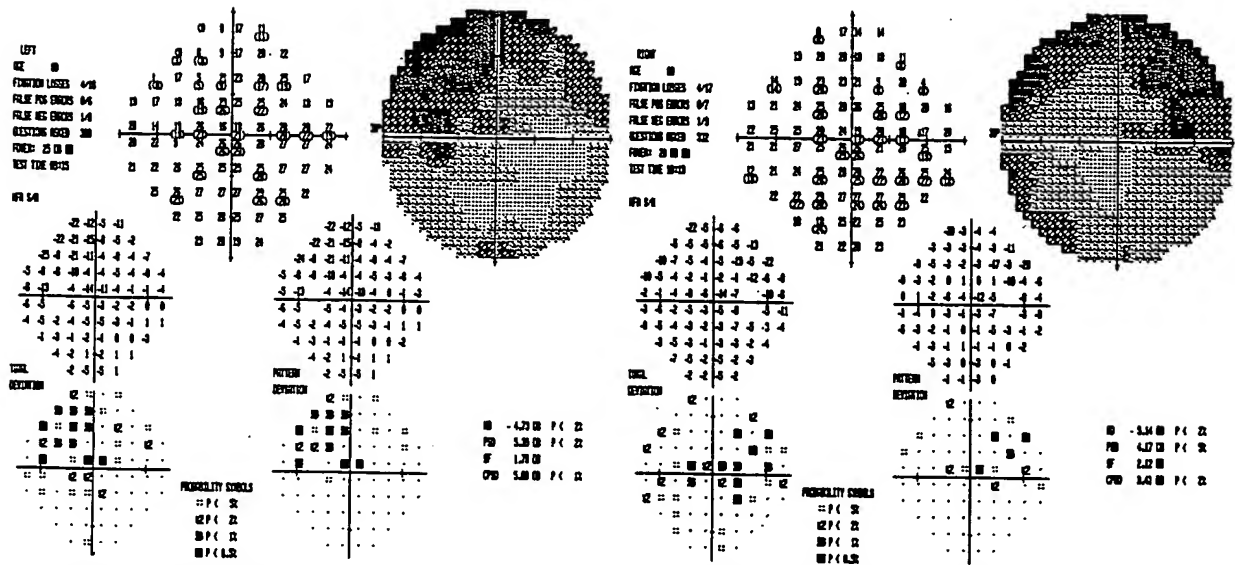


FIG. 6. Case 2: Humphrey (San Leandro, CA) visual field (February 8, 1995), right and left eyes. Note mean deviations of -5.14 dB OD and -4.73 dB OS.

Case 5

A 48-year-old woman had a 2-year history of decreased night vision and peripheral visual loss in the right eye. Medical history included migraines but no evidence of systemic disease. Visual acuity was 20/20 OD and 20/15 OS. Fundus examination of the right eye showed a normal optic nerve with mild vessel attenuation. The left eye was considered normal. Electroretinogram in the right eye was extinguished. The left eye ERG was normal. Visual fields showed moderate constriction of the right eye and full field in the left eye. Fluorescein angiogram was negative. Family history was unremarkable, including that of her twin sister who had no record of visual impairment. Her serum showed an antibody reaction on ELISA, 1:400 against retina and 1:200

against optic nerve (Table 1). Western blot analysis identified an antibody reaction with a unique 22-kDa neuronal antigen, present both in the retina and optic nerve.

Case 6

A 43-year-old woman reported blurry vision in her right eye with no pain on eye movement. Visual acuity was 20/40 OD and 20/20 OS. She had an afferent pupillary defect in the right eye. Biomicroscopy produced normal findings. Her fundus examination showed normal optic nerves and retinas. A magnetic resonance scan, complete blood count, and sedimentation rate were normal. A Lyme disease titer was negative.

More than 1 week later she reported that her vision had decreased significantly with no pain. She had no light

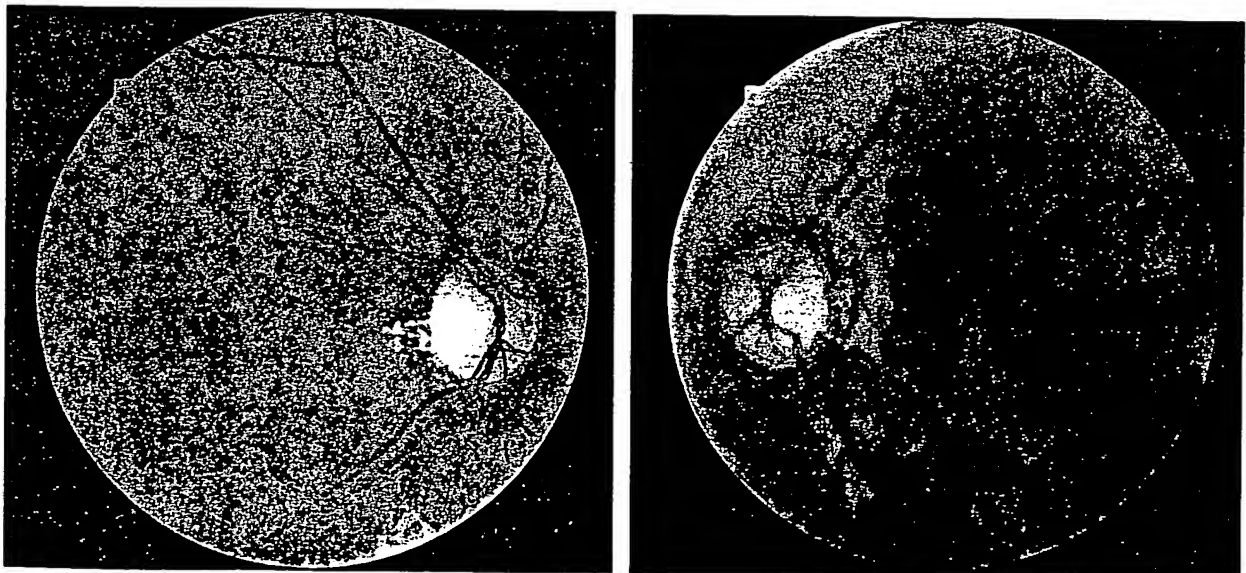


FIG. 7. Case 2: Fundus photograph (February 8, 1995), right eye (on left) and left eye (on right). Note optic nerve pallor in both eyes with narrowing of the blood vessels.

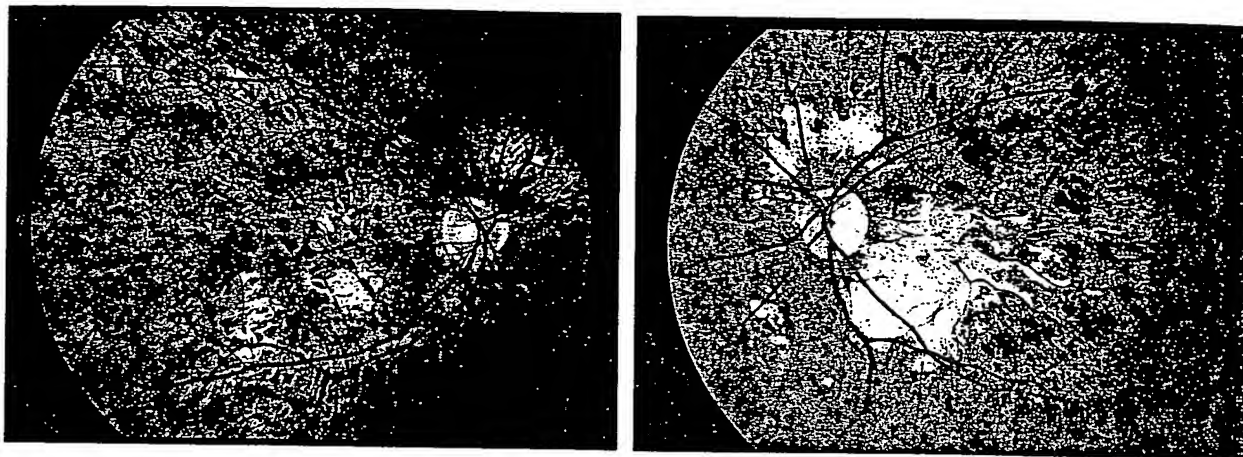


FIG. 8. Case 3: Fundus photograph (January 29, 1992), right eye (on left) and left eye (on right). Note retinal pigment epithelial atrophy and pigment clumping throughout posterior pole, left eye > right eye.

perception, with an amaurotic pupil in the right eye. Findings in biomicroscopic and fundus examination were again normal. One gram intravenous methylprednisolone was prescribed for 4 days. Unfortunately, her vision returned only slightly. A Humphrey visual field examination 6 months later showed a severely depressed field in the right eye but a normal field in the left eye.

An evaluation at 9 months revealed no neurologic or systemic disease. Since her initial episode, she said her vision had improved, then decreased, although she was unsure of the exact time sequence. Both her and her family's medical histories were unremarkable. Her visual acuity was count fingers at 1 ft OD and 20/20 OS. There was a 4+ afferent pupillary defect in the right eye. The Humphrey visual fields in the right eye showed an overall depression with an inferonasal defect; the left eye was normal. Tension applanation pressure was 17 mm Hg OU. The fundus examination in the right eye showed diffuse optic atrophy with loss of the nerve fiber layer

and an otherwise normal macula, retina, and vessels. The left eye was normal (Fig. 10).

An ANA titer of 1:640 with a positive speckle pattern was found. However, anti-double-stranded DNA, cat-scratch testing, cytoplasmic-antineutrophilic cytoplasmic antibody (C-ANCA) titer, Fluorescent treponemal antibody-absorption test, Sjögren antibodies, and Lyme titers were all negative. Serologic analysis of the patient's serum showed an antibody reaction on ELISA, 1:400 against retina and 1:400 against optic nerve (Table 1). Western blot analysis identified an antibody reaction with a unique 22-kDa neuronal antigen, present in the retina and optic nerve. One month later, photopic and scotopic ERGs were normal, although amplitudes were reduced in the affected right eye. Implicit times were normal on all tracings.

Case 7

As previously reported (23), a 46-year old woman was referred for progressive, painless bilateral loss of vision over 12-months. The loss of central vision was accompanied by decreased night vision and photopsia. These were described as flickering and shimmering lights in the central field. Sixteen months earlier she had had a malignant melanoma removed from the right thigh. Nine months after the removal of the tumor, a metastatic lesion was removed from the right inguinal lymph node.

Visual acuity was correctable to only 1/200 OU. Kinetic visual fields on the Goldmann perimeter showed bilateral central scotomas. There was no afferent defect, and color vision showed a mixed deficit. The fundus examination showed moderate bilateral optic nerve pallor and severe attenuation of retinal arteries and veins (Fig. 11). Findings in the remaining neuro-ophthalmologic examination were normal.

The ERG in both eyes showed a slightly attenuated a-wave and an absent b-wave. There was no evidence of oscillatory potentials. This pattern was consistent with congenital stationary night blindness or melanoma-associated retinopathy (MAR) syndrome.

Findings in a complete neurologic and general physical examination were unremarkable. A magnetic reso-



FIG. 9. Case 4: Fundus photograph (October 28, 1992), right eye. Note pigmentation in the periphery.

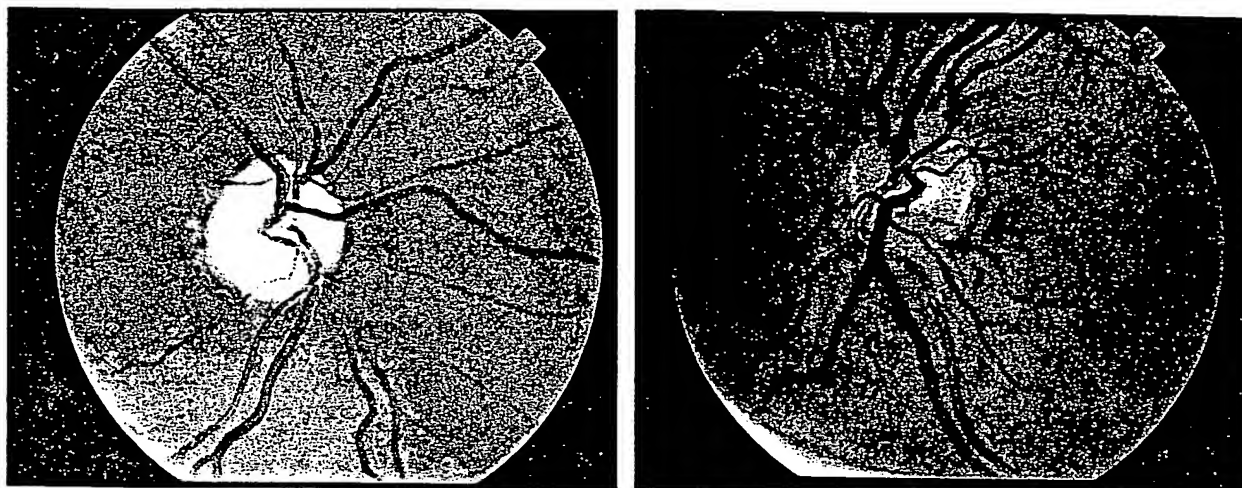


FIG. 10. Case 6: Fundus photograph (February 2, 1995), right and left eyes. Note profound optic atrophy and loss of nerve fiber layer in right eye (on left). Left eye (on right) is normal.

nance scan of the head and orbits produced normal findings. Results of the cerebrospinal fluid examination were normal, and no malignant cells were detected. The patient's serum showed an antibody reaction on ELISA, 1:400 against retina and 1:100 (normal) against optic nerve (Table 1). Western blot analysis identified an antibody reaction with a 22-kDa neuronal antigen, present in the retina only.

Case 8

A 49-year-old woman had a 1.5-year history of visual loss and flashes of light. Visual acuity was 20/60 OD and less than 20/250 OS. Kinetic perimetry using the Goldmann perimeter in the right eye was normal, whereas the left eye showed a central scotoma. The fundus examination showed a slight narrowing of the vessels with wrinkling of the internal limiting membrane and perhaps some macular edema. The right optic nerve was normal, and the left optic nerve showed mild temporal pallor. An ERG performed on April 22, 1990 was abnormal in both eyes. The a-wave was subnormal, and the b-wave was severely reduced and barely recordable (OS > OD).

Eight months later, visual acuity was 20/200 OD and 3/200 OS. Kinetic perimetry on the Goldmann perimeter showed bilateral central scotomas in both eyes, with the scotoma in the left eye being larger than that in the right. The patient's serum showed an antibody reaction on ELISA, 1:400 against retina and 1:200 against optic nerve (Table 1). Western blot analysis identified an antibody reaction with a unique 22-kDa neuronal antigen, present in the retina and optic nerve.

Two years later, visual acuity was 20/100 OD and 20/125 OS. Electroretinogram showed extinguished scotopic responses in both eyes. Flicker fusion was reduced but recordable. Prednisone was prescribed at 20 mg once daily. However, the patient discontinued the prednisone after 1 week and thought her vision had worsened.

Two years later, visual acuity was 20/200 OD and 20/200 OS. Central scotomas were demonstrated with kinetic perimetry on the Goldmann perimeter. The fun-

dus examination revealed mild to moderate optic pallor of the right eye and moderate temporal pallor of the left optic nerve (Fig. 12). The macula also showed focal yellow deposits.

The patient's medical history remained unremarkable. Syphilitic serology was negative. Vitamin A and carotin levels were normal. Of note, the patient's daughter had had malignant melanoma, and another daughter had had a precancerous lesion.

DISCUSSION

Autoimmune syndromes have recently been described with CAR syndrome (1-21) and MAR syndrome (23-27). Mizener et al. (28) recently described autoimmune retinopathy in the absence of cancer. They reported two patients with a strong family history of autoimmune disease who had severe monocular visual loss with photopsia, ring scotoma, and abnormal ERGs, despite a normal-appearing fundus. Both patients' sera had antiretinal antibodies that specifically labeled the inner plexiform layer of the retina by indirect immunoperoxidase testing (28). Heckenlively et al. (29) and Aptsiauri et al. (30) have recently reported patients with progressive parretinal degeneration from such conditions as idiopathic retinopathy and retinitis pigmentosa to have CAR-like clinical changes in association with the presence of antibodies to recoverin and other retinal proteins.

Whitcup et al. (31) have recently reported a group of patients with a condition they call recoverin-associated retinopathy syndrome. They demonstrated a cellular immune response against recoverin in one patient who had no history of cancer but who experienced retinal degeneration clinically similar to patients with CAR. Elevated titers of antibodies against recoverin were also found in this patient and in one of three patients with retinal degeneration, but in none of eight patients with uveitis. Serum from the patient with the disease resembling CAR produced immunostaining of the rods, cones, outer plexiform layer, and some cone bipolar cells. However, the

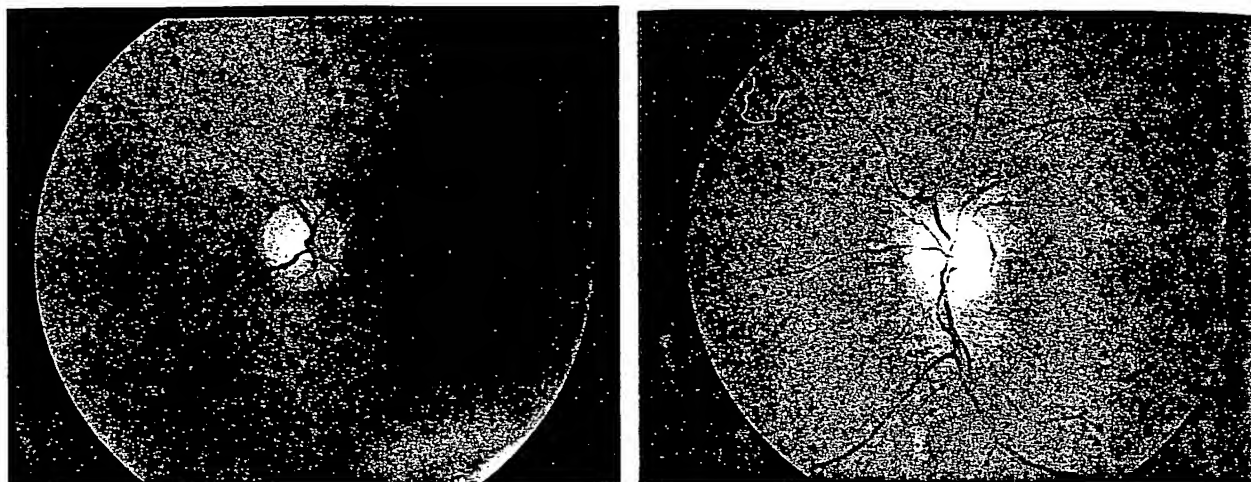


FIG. 11. Case 7: Fundus photograph (January 23, 1992), right eye (on left) and left eye (on right). Note optic atrophy in both eyes with narrowing of the blood vessels.

serum from patients with uveitis or other retinal degeneration did not show specific reactivity with the retina.

In an editorial accompanying the article by Whitcup et al. (32), we discussed CAR in comparison with recovering-associated retinopathy, and the importance of separating CAR from other autoimmune retinopathies that may have a similar presentation. Unfortunately, this may not be a simple procedure. Appropriate clinical history and physical, ophthalmologic examination, laboratory, and electrophysiologic examinations, in conjunction with the appropriate immunologic testing, are necessary to diagnose the CAR syndrome and separate it from other noncancer-associated retinopathies including those retinopathies associated with the 22-kDa antigen. Recently, Peek et al. (33) described Müller cell-specific autoantibodies against a 35-kDa retinal antigen in a patient with progressive visual loss over 4 years and no evidence of cancer. They also recognized the need for further research to identify whether these antibodies are an epiphenomenon or are involved in the pathogenesis of their patients' retinopathy.

Antiretinal antibodies are possibly an epiphenomenon in response to tissue damage in nonspecific mechanisms.

For example, antiretinal antibodies have been described in retinal degeneration (29,30), retinitis pigmentosa (34–36), age-related macular degeneration (37), and cone dystrophy (38). However, the role of autoantibodies in these retinal diseases has not been established. Although we have also found various conditions that may show diffuse immunologic reaction against retina and optic nerve, we have now identified a subgroup of patients with an antibody reaction to a 22-kDa protein present in the retina and optic nerve. Although there are an increasing number of reported retinopathy-associated retinal antigens, we are not aware of any reports describing reactions to the 22-kDa antigen.

In this report we describe eight patients with abnormal antibody reactions involving the retina and optic nerve. Western blot reactions identified an immunologic commonality involving a 22-kDa protein present in retina and optic nerve. Case 7 was exceptional in that the 22-kDa antigen-antibody reaction was confined to the retina and was not evident in the optic nerve, indicating that a separate protein was involved that exhibited a coincidental reaction in Western blot testing on retina. All of the patients had either an abnormal ERG or optic atrophy.

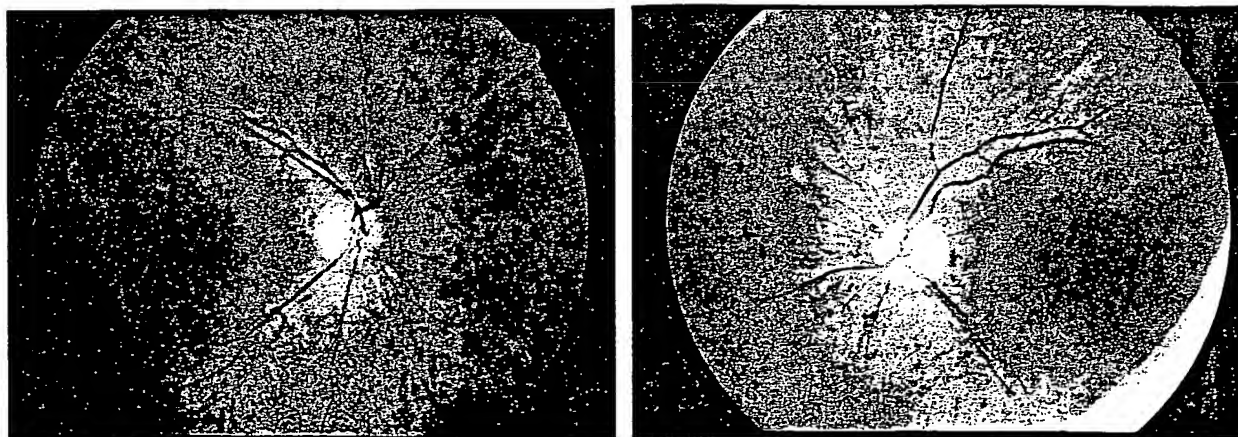


FIG. 12. Case 8: Fundus photograph (June 2, 1995), right eye (on left) and left eye (on right). Note the optic pallor in both eyes.

Six patients had both. Seven of the eight patients were women. Seven had ERG abnormalities varying from mild to severe. Six had optic atrophy, but only one of these had a recognized systemic illness, a history of metastatic cutaneous malignant melanoma (23). No other patient was found to have cancer. These patients had in common unexplained visual loss from retinal or optic nerve disease.

The fact that two of our patients had only one eye affected (cases 5 and 6) should not exclude autoimmune implications. Mizener et al. (28) reported two patients with possible autoimmune retinopathy who had only one eye involved initially. Their first patient had no symptoms in the right eye after 5 years of progressive visual loss in the left eye. However, the ERG showed subclinical evidence of disease in the right eye. Their second patient had a long, stuttering course of monocular vision loss and was symptomatic in the right eye for 19 years before the left eye became symptomatic. Electoretinography again confirmed that he was affected bilaterally, even though the ERG had been normal in the left eye previously. The authors thought the ERG was more sensitive than perimetry in identifying the retinopathy. Other autoimmune diseases also affect one eye or one optic nerve. Dysfunction of immunoregulation is considered to play a central role in the pathogenesis of multiple sclerosis, a disorder in which monocular optic neuritis is common (39-42).

There were some remarkable clinical features among the patients with sera reacting to the 22-kDa antigen. The patient in case 1 has been observed and evaluated extensively, and neither systemic illness nor cancer has developed. This patient is unique in that her retinopathy and optic neuropathy progressed for 10 years, when she was finally stabilized by combined therapy with cyclosporine, azathioprine, and prednisone. Patient 3 had massive geographic atrophy centrally. Patient 4 was a child who had probable viral neuroretinitis with a completely flat ERG and 22-kDa antigens found consistently for several years after the initial insult. However, the patients in cases 1, 2, 5, and 8 had more subtle retinal findings with progressive visual loss over an extended period. Patient 5 was also interesting because of a primary retinal abnormality with a normal-appearing fundus, essentially normal visual acuity with a flat ERG in the right eye, and normal acuity and ERG in the left eye. Patient 6 had a normal ERG, with some reduction in amplitude in the involved eye and had a retrobulbar optic neuritis-type of presentation with progression of vision to no light perception in the involved eye that never improved. Finally, patient 7 was the only one of the eight known to have cancer, found to be cutaneous melanoma. This patient was thought to have MAR syndrome, but we have not found the 22-kDa reaction in other patients with MAR syndrome (23-27). Although these cases are different, they share a common reaction to the 22-kDa retinal antigen.

The proximity of the 23-kDa cancer-associated antigen to the 22-kDa idiopathic retinopathy antigen in Western blot analyses led us to compare immunologic

and clinical characteristics of the two groups. Optic neuropathy, but not retinopathy, has also been reported in cancer patients, occurring primarily in association with small-cell carcinoma of the lung, but it has also been seen in leukemias and lymphomas (43-46). The 23-kDa CAR antigen is located within the retinal photoreceptor cells with some reports of its distribution within bipolar cells, optic nerve, and the pineal body (47-50). Although the 22-kDa antigen can be clearly shown in retina and optic nerve, the extent of its distribution throughout the central nervous system has yet to be determined. This retinal and optic nerve distribution corresponds with the clinical picture of mild to severe optic atrophy and retinal abnormalities in six of our seven patients.

Differences in the intensity of related immunologic reactions of the two groups are apparent, in that antibody titers of patients with 23-kDa CAR commonly exceed 1:1000, whereas the patients with the 22-kDa antigen do not surpass 1:1000. Perhaps the lower titers reflect why some of the patients described in this study had a much slower progression of visual loss over several years, similar to the patients reported by Mizener et al. (28) and Peek et al. (33).

In summary, we describe eight patients with unexplained loss of vision who produce autoantibodies reactive with a low-molecular-mass 22-kDa neuronal antigen present within the retina and/or optic nerve. Seven of the eight had an antibody reaction to both retina and optic nerve, whereas the exception, Case 7, may have a reaction with an entirely different protein. Seven of the eight patients had ERG abnormalities varying from mild to severe. Six displayed features of optic atrophy. One patient had progressive visual loss over 10 years that was apparently stabilized by the use of immunosuppressive therapy.

It is premature to suggest autoimmunity as a causative factor in the progression of vision loss in the 22-kDa group because the elevated antibody titers may reflect evidence of the immune system's response to the non-specific destruction of the retina and optic nerve. However, this novel immunologic marker could serve to identify those who may benefit from further immunologic investigation, and possibly immunologic therapy. As we mentioned in our recent editorial (32), only one commercial laboratory test is available to test for the 23-kDa CAR retinal antigen. Unfortunately, to separate the diagnosis of CAR syndrome from other types of possible autoimmune-related retinopathy, more extensive immunologic, ophthalmologic, and electrophysiologic testing is necessary. The understanding of these important retinopathies may show them to be related entities, and research in this field is rapidly evolving.

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REFERENCES

- Thirkill CE, Tait RC, Tyler NK, Roth AM, Keltner JL. Intraperitoneal cultivation of small-cell carcinoma induces expression of the retinal cancer-associated retinopathy antigen. *Arch Ophthalmol* 1993;111:974-8.
- Thirkill CE, Tait RC, Tyler NK, Roth AM, Keltner JL. The Cancer connection: An antigen immunologically related to the retinal CAR antigen is expressed in small cell carcinoma of the lung. In: J.P. Demouchamps, ed. *Proceedings of the Third International Symposium on Uveitis*. Amsterdam: Kugler Publications 1993;133-5.
- Keltner JL, Roth AM, Chang RS. Photoreceptor degeneration. Possible autoimmune disorder. *Arch Ophthalmol* 1983;101:564-9.
- Thirkill CE, Roth AM, Keltner JL. Cancer-associated retinopathy. *Arch Ophthalmol* 1987;105:372-5.
- Sawyer RA, Selhorst JB, Zimmerman LE, Hoyt WF. Blindness caused by photoreceptor degeneration as a remote effect of cancer. *Am J Ophthalmol* 1976;81:606-13.
- Korneguth SE. Neuronal proteins and paraneoplastic syndromes. *N Engl J Med* 1989;321:1607-8.
- Korneguth SE, Klein R, Appen R, Choate J. Occurrence of anti-retinal ganglion cell antibodies in patients with small cell carcinoma of the lung. *Cancer* 1982;50:1289-93.
- Korneguth SE, Kalinke T, Grunwald GB, Schutta H, Dahl D. Anti-neurofilament antibodies in the sera of patients with small cell carcinoma of the lung and with visual paraneoplastic syndrome. *Cancer Res* 1986;46:2588-95.
- Klinge TG, Burde RM, Rappazzo JA, Isserman MJ, Burgess D, Kantor O. Paraneoplastic retinopathy. *J Clin Neuro-Ophthalmol* 1984;4:239-45.
- Thirkill CE, FitzGerald P, Sergott RC, Roth AM, Tyler NK, Keltner JL. Cancer-associated retinopathy (CAR syndrome) with antibodies reacting with retinal, optic-nerve, and cancer cells. *N Engl J Med* 1989;321:1589-94.
- Thirkill CE, Roth AM, Keltner JL. Paraneoplastic retinopathy syndrome. *Ophthalmology* 1993;100:147.
- Thirkill CE, Keltner JL, Tyler NK, Roth AM. Antibody reactions with retina and cancer-associated antigens in 10 patients with cancer-associated retinopathy. *Arch Ophthalmol* 1993;111:931-7.
- Cogan DG, Kuwabara T, Currie J, Kattah J. Paraneoplastic retinopathy simulating cone dystrophy with achromatopsia. *Klin Monatsbl Augenheilkd* 1990;197:156-8.
- Jacobson DM, Thirkill CE. Paraneoplastic cone dysfunction: An unusual visual remote effect of cancer. *Arch Ophthalmol* 1996;113:1580-2.
- Anderson NE. Paraneoplastic optic neuritis and external ophthalmoplegia. *Aust N Z J* 1987;17:539.
- Sergott RC, Brown MJ, Silberberg DH, Lisak RP. Antigalactocerebroside serum demyelinate optic nerve in vivo. *Neurol Sci* 1984;64:297-303.
- Boghen DR, Sebag M, Michaud J. Paraneoplastic optic neuritis and encephalomyelitis. *Arch Neurol* 1988;45:353-6.
- Thirkill CE. Cancer Associated Retinopathy, The CAR Syndrome. *Neuro-Ophthalmology* 1994;14:297-323.
- Keltner JL, Thirkill CE, Tyler NK, Roth AM. Management and monitoring of cancer associated retinopathy (CAR). *Arch Ophthalmol* 1992;110:48-53.
- Thirkill CE, Tait RC, Tyler NK, Roth AM, Keltner JL. The Cancer-Associated Retinopathy is a recoverin-like protein. *Invest Ophthalmol Vis Sci* 1992;33:2768-72.
- Jacobson DM, Thirkill CE, Tipping SJ. A clinical trial to diagnose paraneoplastic retinopathy. *Ann Neurol* 1990;28:162-7.
- Hooper PL, Kaplan HJ. Triple agent immunosuppression in ser-piginous choroiditis. *Ophthalmology* 1991;98:944-52.
- Weinstein J, Bresnick G, Korneguth SE. Paraneoplastic retinopathy associated with anti-retinal bipolar cell antibodies in cutaneous malignant melanoma. *Ophthalmology* 1994;101:1236-43.
- Alexander KR, Fishman GA, Peachey NS, Marchese AL, Tso MO. 'On' response defect in paraneoplastic night blindness with cutaneous malignant melanoma. *Invest Ophthalmol Vis Sci* 1992;33:477-83.
- Berson EL, Lessell S. Paraneoplastic night blindness with malignant melanoma. *Am J Ophthalmol* 1988;106:307-11.
- Milam AH, Saari JC, Jacobson SG, Lubinski WP, Feun LG, Alexander KR. Autoantibodies against retinal bipolar cells in cutaneous melanoma-associated retinopathy. *Invest Ophthalmol Vis Sci* 1993;34:91-100.
- Okel BB, Thirkill CE, Anderson K. An unusual case of melanoma-associated retinopathy. *Ocular Immunol Inflamm* 1995;3:121-7.
- Mizener JB, Kimura AE, Adamus G, Thirkill C, Goeken JA, Kardon RH. Autoimmune retinopathy in the absence of cancer. *Am J Ophthalmol* 1997;123:607-18.
- Heckenlively JR, Oversier JJ, Jordan B, Aptsiauri N. CAR-like syndrome: Progressive paraneoplastic degenerations not associated with cancer but with anti-recoverin (and other) anti-retinal antibodies (abstract 997). *Invest Ophthalmol Vis Sci* 1998;39:S216.
- Aptsiauri N, Jordan BI, Heckenlively J. Anti-recoverin immunoreactivity in patients with retinal degeneration without systemic malignancy (abstract 1694). *Invest Ophthalmol Vis Sci* 1998;39:S363.
- Whitcup SM, Vistica BP, Milam AH et al. Recoverin-associated retinopathy: A clinically and immunologically distinctive disease. *Am J Ophthalmol* 1998;126:230-7.
- Keltner JL, Thirkill CE. Cancer associated retinopathy vs. recoverin associated retinopathy. *Am J Ophthalmol* 1998;126:296-302.
- Peek R, Verbraak F, Coevoet HMN, Kijlstra A. Muller cell-specific autoantibodies in a patient with progressive loss of vision. *Invest Ophthalmol Vis Sci* 1998;39:1976-7.
- Kumar M, Gupta RM, Nema HV. Role of autoimmunity in retinitis pigmentosa. *Ann Ophthalmol* 1983;15:838-40.
- Chant SM, Heckenlively JR, Meyers-Elliott RH. Autoimmunity in hereditary retinal degeneration. I: basic studies. *Br J Ophthalmol* 1985;69:758-64.
- Thirkill CE, Roth AM, Takemoto DJ, Tyler NK, Keltner JL. Antibody indications of secondary and superimposed retinal hypersensitivity in retinitis pigmentosa. *Am J Ophthalmol* 1991;112:132-7.
- Gurne DH, Tso MOM, Edward DP, Ripps H. Antiretinal antibodies in serum of patients with age-related macular degeneration. *Ophthalmology* 1991;98:602-7.
- Isashiki Y, Ohba N, Nakagawa M, Miyake Y. Antibodies against human retinal proteins in serum from patients with cone dystrophy. *Jpn J Ophthalmol* 1992;36:323-30.
- Oksaranta O, Tarvonen S, Ilonen J, et al. T-cell subpopulations in the cerebrospinal fluid and peripheral blood of patients with multiple sclerosis: a follow-up study. *Neurology* 1996;47:1542-5.
- Paty DW, Li DKB, the UBC MS/MRI Study Group, IFN- β Multiple Sclerosis Study Group. Interferon β -1b is effective in relapsing-remitting multiple sclerosis: I. Clinical results of a multicenter, randomized, double-blind, placebo-controlled trial. *Neurology* 1993;43:655-61.
- Hohlfeld R, Meinl E, Weber F, et al. The role of autoimmune T lymphocytes in the pathogenesis of multiple sclerosis. *Neurology* 1995;45(suppl 6):S33-8.
- Lisak RP. Overview of the rationale for immunomodulating therapies in multiple sclerosis. *Neurology* 1988;38(suppl):5-8.
- Sergott RC, Brown MJ, Polenta RM, Lisak RP, Silberberg DH. Optic nerve demyelination induced by human serum: Patients with

- multiple sclerosis or optic neuritis and normal subjects. *Neurology* 1985;35:1438-42.
44. Anderson NE, Cunningham JM, Posner JB. Autoimmune pathogenesis of paraneoplastic neurological syndromes. *Crit Rev Neurobiol* 1987;3:245-99.
45. Boghen D, Sebag M, Michaud J. Paraneoplastic optic neuritis and encephalomyelitis. Report of a case. *Arch Neurol* 1988;45:353-6.
46. Luiz JE, Lee AG, Keltner JL, Thirkill CE, Lai EC. Paraneoplastic optic neuropathy and autoantibody production in small-cell carcinoma of the lung. *J Neuro-Ophthalmol* 1998;18:178-81.
47. Milam AH, Dacey DM, Dizhoor AM. Recoverin immunoreactivity in mammalian cone bipolar cells. *Vis Neurosci* 1993;10:1-12.
48. Igal G, Chanaud NP, Anglade E. Recoverin is highly uveitogenic in Lewis rats. *Invest Ophthalmol Vis Sci* 1994;35:3342-5.
49. Polans AS, Burton MD, Halet TL, Crabb JW, Palczewski K. Recoverin, but not visinin, is an autoantigen in the human retina identified with a cancer-associated retinopathy. *Invest Ophthalmol Vis Sci* 1993;34:81-90.
50. Thirkill CE. Retinal antibodies. In: Peter JB, Shoenfeld Y, eds. *Autoantibodies*. The Netherlands: Elsevier, 1996:694-9.